

FIG. 1

sequence\_AA\_2D12.5\_variable domains.txt

```
>2D12.5VL_MOUSE
(1) QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGNNRPPGVPARFSGSLIGDKAALTIAGTQTED
    EAIYFCALWYSNHWVFEGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWV

>2D12.5VH_MOUSE
(5) QVKLQESGPGGLVQPSQSL SITCTVSGFSLTDYGVHWVRQSPGKGLEWLGVIWSGGGTAYTAAFISRLNIY
    KDNSKNQVFFEMNSLQANDTAMYVCARRGSYPYNYFDVWGQGTTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAFIS
(8) CDR3 - RGSYPYNYFDV
```

FIG. 2

## Translation of 2D12.5 VH variable genes

|      |                            |     | 10   | 20 | 30 | 40 | 50  |     |
|------|----------------------------|-----|--|----|----|----|-----|-----|
| (9)  | 2d12.5 VH native hybridoma | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     |     |
| (10) | 2d12.5 VH native cloned    | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 50  |
| (11) | 2d12.5 VH N87D_cloned      | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 50  |
| (12) | 2d12.5 VH N87D_G53C_cloned | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 50  |
| (13) | 2d12.5 VH_N87D_G54C_cloned | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 50  |
| (14) | 2d12.5 VH_N87D_G55C_cloned | 1   | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 50  |
|      |                            |     | 60   | 70 | 80 | 90 | 100 |     |
|      | 2d12.5 VH native hybridoma | 51  | WSGGGTAYTAAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYYCARRGSY  |    |    |    |     | 100 |
|      | 2d12.5 VH native cloned    | 51  | ..... ..... ..... ..... ..... ..... ..... .....      |    |    |    |     | 100 |
|      | 2d12.5 VH N87D_cloned      | 51  | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | D  |     | 100 |
|      | 2d12.5 VH N87D_G53C_cloned | 51  | ..C..... ..... ..... ..... ..... ..... ..... .....   |    |    | D  |     | 100 |
|      | 2d12.5 VH_N87D_G54C_cloned | 51  | ...C..... ..... ..... ..... ..... ..... ..... .....  |    |    | D  |     | 100 |
|      | 2d12.5 VH_N87D_G55C_cloned | 51  | ....C..... ..... ..... ..... ..... ..... ..... ..... |    |    | D  |     | 100 |
|      |                            |     | 110  |    |    |    |     |     |
|      | 2d12.5 VH native hybridoma | 101 | PYNYFDVWGQGTTTVTVSS                                  |    |    |    |     | 118 |
|      | 2d12.5 VH native cloned    | 101 | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | A  |     | 118 |
|      | 2d12.5 VH N87D_cloned      | 101 | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | A  |     | 118 |
|      | 2d12.5 VH N87D_G53C_cloned | 101 | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | A  |     | 118 |
|      | 2d12.5 VH_N87D_G54C_cloned | 101 | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | A  |     | 118 |
|      | 2d12.5 VH_N87D_G55C_cloned | 101 | ..... ..... ..... ..... ..... ..... ..... .....      |    |    | A  |     | 118 |

FIG. 3A

## 2D12.5 VH variable genes

|                                 |     |  |     |     |     |     |     |  |
|---------------------------------|-----|--|-----|-----|-----|-----|-----|--|
|                                 |     |  | 10  | 20  | 30  | 40  | 50  |  |
| (15) 2d12.5 VH native hybridoma | 1   | GTGAAGCTGCAGGAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGAGCCT   | 50  |     |     |     |     |  |
| (16) 2d12.5 VH native cloned    | 1   | .....T.....  | 50  |     |     |     |     |  |
| (17) 2d12.5 VH N87D_cloned      | 1   | .....T.....  | 50  |     |     |     |     |  |
| (18) 2d12.5 VH N87D_G53C_cloned | 1   | .....T.....  | 50  |     |     |     |     |  |
| (19) 2d12.5 VH N87D_G54C_cloned | 1   | .....T..G.....                                       | 50  |     |     |     |     |  |
| (20) 2d12.5 VH N87D_G55C_cloned | 1   | .....T.....  | 50  |     |     |     |     |  |
|                                 |     |  | 60  | 70  | 80  | 90  | 100 |  |
| 2d12.5 VH native hybridoma      | 51  | GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATGGTGTAC   | 100 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 51  | .....  | 100 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 51  | .....  | 100 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 51  | .....  | 100 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 51  | .....  | 100 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 51  | .....  | 100 |     |     |     |     |  |
|                                 |     |  | 110 | 120 | 130 | 140 | 150 |  |
| 2d12.5 VH native hybridoma      | 101 | ACTGGGTTCCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGAGTGATA  | 150 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 101 | .....  | 150 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 101 | .....  | 150 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 101 | .....  | 150 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 101 | .....  | 150 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 101 | .....  | 150 |     |     |     |     |  |
|                                 |     |  | 160 | 170 | 180 | 190 | 200 |  |
| 2d12.5 VH native hybridoma      | 151 | TGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTTCATATCCAGACTGAA  | 200 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 151 | .....  | 200 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 151 | .....  | 200 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 151 | .....T.....  | 200 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 151 | .....T..T.....                                       | 200 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 151 | .....T.....  | 200 |     |     |     |     |  |
|                                 |     |  | 210 | 220 | 230 | 240 | 250 |  |
| 2d12.5 VH native hybridoma      | 201 | CATCTACAAGGACAAATTCGAAGAACCAAGTTTTCTTTGAAATGAACAGTC  | 250 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 201 | .....  | 250 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 201 | .....  | 250 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 201 | .....  | 250 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 201 | .....  | 250 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 201 | .....  | 250 |     |     |     |     |  |
|                                 |     |  | 260 | 270 | 280 | 290 | 300 |  |
| 2d12.5 VH native hybridoma      | 251 | TGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGTAGCTAC   | 300 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 251 | .....  | 300 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 251 | .....G.....  | 300 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 251 | .....G.....  | 300 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 251 | .....G.....  | 300 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 251 | .....G.....  | 300 |     |     |     |     |  |
|                                 |     |  | 310 | 320 | 330 | 340 | 350 |  |
| 2d12.5 VH native hybridoma      | 301 | CCTTACAACCTACTTCGATGTCTGGGGCCAAGGGACCACAGTCCACCGTCTC | 350 |     |     |     |     |  |
| 2d12.5 VH native cloned         | 301 | .....G.....  | 350 |     |     |     |     |  |
| 2d12.5 VH N87D_cloned           | 301 | .....G.....  | 350 |     |     |     |     |  |
| 2d12.5 VH N87D_G53C_cloned      | 301 | .....G.....  | 350 |     |     |     |     |  |
| 2d12.5 VH N87D_G54C_cloned      | 301 | .....G.....  | 350 |     |     |     |     |  |
| 2d12.5 VH N87D_G55C_cloned      | 301 | .....G.....  | 350 |     |     |     |     |  |

FIG. 3B

2D12.5 VH variable genes

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      ....
2d12.5 VH native hybridoma 351 CTCA 354
2d12.5 VH native cloned   351 .G.. 354
2d12.5 VH N87D_cloned     351 .G.. 354
2d12.5 VH N87D_G53C_cloned 351 .G.. 354
2d12.5 VH_N87D_G54C_cloned 351 .G.. 354
2d12.5 VH N87D_G55C_cloned 351 .G.. 354
```

FIG. 4

## Translation of 2D12.5 VL genes

|      |                            |     | 10  | 20 | 30 | 40 | 50 |     |
|------|----------------------------|-----|---|----|----|----|----|-----|
| (21) | 2d12.5 VL native hybridoma | 1   | ..... ..... ..... ..... ..... ..... ..... .....     |    |    |    |    |     |
|      |                            |     | AVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLEFTGLIG |    |    |    |    | 50  |
| (22) | 2d12.5 VL native cloned    | 1   | ..... ..... ..... ..... ..... ..... ..... .....     |    |    |    |    | 50  |
| (23) | 2d12.5 VL N53C_cloned      | 1   | ..... ..... ..... ..... ..... ..... ..... .....     |    |    |    |    | 50  |
|      |                            |     |   | 60 | 70 | 80 | 90 | 100 |
|      | 2d12.5 VL native hybridoma | 51  | GNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG  |    |    |    |    | 100 |
|      | 2d12.5 VL native cloned    | 51  | ..... ..... ..... ..... ..... ..... ..... .....     |    |    |    |    | 100 |
|      | 2d12.5 VL N53C_cloned      | 51  | .C..... ..... ..... ..... ..... ..... ..... .....   |    |    |    |    | 100 |
|      |                            |     | ..... .....   |    |    |    |    |     |
|      | 2d12.5 VL native hybridoma | 101 | GGTRLTVLG   |    |    |    |    | 109 |
|      | 2d12.5 VL native cloned    | 101 | ...K....S   |    |    |    |    | 109 |
|      | 2d12.5 VL N53C_cloned      | 101 | ...K....S   |    |    |    |    | 109 |

## FIG. 5

|      |                            |     |  |     |     |     |     |     |     |
|------|----------------------------|-----|--|-----|-----|-----|-----|-----|-----|
|      |                            |     |  | 10  | 20  | 30  | 40  | 50  |     |
| (24) | 2d12.5 VL native hybridoma | 1   | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
| (25) | 2d12.5 VL native cloned    | 1   | GCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGT                   |     |     |     |     |     | 50  |
| (26) | 2d12.5 VL N53C_cloned      | 1   | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 50  |
|      |                            |     |  | 60  | 70  | 80  | 90  | 100 |     |
|      | 2d12.5 VL native hybridoma | 51  | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 51  | CACACTCACTTGTCTCGCTCAAGTACTGGGGCTGTTACGACTAGTAACTATG                 |     |     |     |     |     | 100 |
|      | 2d12.5 VL N53C_cloned      | 51  | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 100 |
|      |                            |     |  | 110 | 120 | 130 | 140 | 150 |     |
|      | 2d12.5 VL native hybridoma | 101 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 101 | CCAAGTGGGTCCAAGAGAAACCAGATCATTATTCTGCTCTAATAGGT                      |     |     |     |     |     | 150 |
|      | 2d12.5 VL N53C_cloned      | 101 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 150 |
|      |                            |     |  | 160 | 170 | 180 | 190 | 200 |     |
|      | 2d12.5 VL native hybridoma | 151 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 151 | GGTAATAATAACCGACCTCCAGGTGTTCTCTGCCAGATTCTCAGGCTCCCT                  |     |     |     |     |     | 200 |
|      | 2d12.5 VL N53C_cloned      | 151 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 200 |
|      |                            |     | ...TG..... ..... ..... ..... ..... ..... ..... ..... ..... .....     |     |     |     |     |     | 200 |
|      |                            |     |  | 210 | 220 | 230 | 240 | 250 |     |
|      | 2d12.5 VL native hybridoma | 201 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 201 | GATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTGAGGATG                   |     |     |     |     |     | 250 |
|      | 2d12.5 VL N53C_cloned      | 201 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 250 |
|      |                            |     |  | 260 | 270 | 280 | 290 | 300 |     |
|      | 2d12.5 VL native hybridoma | 251 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 251 | AGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTGTTTCGGT                  |     |     |     |     |     | 300 |
|      | 2d12.5 VL N53C_cloned      | 251 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     | 300 |
|      |                            |     |  | 310 | 320 |     |     |     |     |
|      | 2d12.5 VL native hybridoma | 301 | ..... ..... ..... ..... ..... ..... ..... ..... ..... .....          |     |     |     |     |     |     |
|      | 2d12.5 VL native cloned    | 301 | GGAGGAACCAGACTGACTGTCCTAGGC  |     |     |     |     |     | 327 |
|      | 2d12.5 VL N53C_cloned      | 301 | ..G.....A..... ..... ..... ..... ..... ..... ..... ..... ..... ..... |     |     |     |     |     | 327 |
|      |                            |     | ..G.....A..... ..... ..... ..... ..... ..... ..... ..... ..... ..... |     |     |     |     |     | 327 |

FIG. 6

## Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

|                                     |     |              |  |     |     |     |     |     |
|-------------------------------------|-----|--------------|--|-----|-----|-----|-----|-----|
|                                     |     |              | 10   | 20  | 30  | 40  | 50  |     |
| (27) 2dVL-TTCL native_cloned        | 1   | RS           | AVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGL | 50  |     |     |     |     |
| (28) 2dVL-TTCL N53C_cloned          | 1   | .....        |  |     |     |     |     | 50  |
| (29) 2d12.5 VL native hybridoma     | 1   | .....        |  |     |     |     |     | 48  |
| (30) TTCL template for gene assembl | 1   | -----        |  |     |     |     |     | ]   |
|                                     |     |              | 60   | 70  | 80  | 90  | 100 |     |
| 2dVL-TTCL native_cloned             | 51  | IGGN         | NNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWV   | 101 |     |     |     |     |
| 2dVL-TTCL N53C_cloned               | 51  | ...C.....    |  |     |     |     |     | 101 |
| 2d12.5 VL native hybridoma          | 49  | .....        |  |     |     |     |     | 99  |
| TTCL template for gene assembl      | 1   | -----        |  |     |     |     |     | ]   |
|                                     |     |              | 110  | 120 | 130 | 140 | 150 |     |
| 2dVL-TTCL native_cloned             | 101 | FGGG         | TKLTVLSRTVAAPSVFIFPPPSDEQLKSGTASVVCLLNNFYPREAKV  | 151 |     |     |     |     |
| 2dVL-TTCL N53C_cloned               | 101 | .....        |  |     |     |     |     | 151 |
| 2d12.5 VL native hybridoma          | 99  | .....R.....G |  |     |     |     |     | 101 |
| TTCL template for gene assembl      | 1   | -----        |  |     |     |     |     | 39  |
|                                     |     |              | 160  | 170 | 180 | 190 | 200 |     |
| 2dVL-TTCL native_cloned             | 151 | QWKVD        | NALQSGNSQESVTEQDSKDYSLSTLTLSKADYKHKVYACEV        | 201 |     |     |     |     |
| 2dVL-TTCL N53C_cloned               | 151 | .....        |  |     |     |     |     | 201 |
| 2d12.5 VL native hybridoma          |     | .....        |  |     |     |     |     |     |
| TTCL template for gene assembl      | 40  | .....        |  |     |     |     |     | 89  |
|                                     |     |              | 210  | 220 |     |     |     |     |
| 2dVL-TTCL native_cloned             | 201 | THQGLSLPVT   | KSFNRGEC*F*                                      | 221 |     |     |     |     |
| 2dVL-TTCL N53C_cloned               | 201 | .....        | *.*  | 221 |     |     |     |     |
| 2d12.5 VL native hybridoma          |     | .....        |  |     |     |     |     |     |
| TTCL template for gene assembl      | 90  | .....        |  | 107 |     |     |     |     |

FIG. 7A

## Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

|      |                              |     |  |     |     |     |  |
|------|------------------------------|-----|--|-----|-----|-----|--|
|      |                              | 10  | 20   | 30  | 40  | 50  |  |
| (31) | 2dVL-TTCL native_cloned      | 1   | AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGA | 50  |     |     |  |
| (32) | 2dVL-TTCL N53C_cloned        | 1   | .....  | 50  |     |     |  |
| (33) | 2d12.5 VL native hybridoma   | 1   | -----  | 44  |     |     |  |
| (34) | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 60  | 70   | 80  | 90  | 100 |  |
|      | 2dVL-TTCL native_cloned      | 51  | AACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTA | 100 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 51  | .....  | 100 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 45  | .....  | 94  |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 110 | 120  | 130 | 140 | 150 |  |
|      | 2dVL-TTCL native_cloned      | 101 | ACTATGCCAACTGGGTCCAAGAGAAACCAGATCATTATTCTACTGGTCTA | 150 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 101 | .....  | 150 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 95  | .....  | 144 |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 160 | 170  | 180 | 190 | 200 |  |
|      | 2dVL-TTCL native_cloned      | 151 | ATAGGTGGTAATAATAACCGACCTCCAGGTGTTCTGCCAGATTCTCAGG  | 200 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 151 | .....TG.....                                       | 200 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 145 | .....  | 194 |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 210 | 220  | 230 | 240 | 250 |  |
|      | 2dVL-TTCL native_cloned      | 201 | CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG | 250 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 201 | .....  | 250 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 195 | .....  | 244 |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 260 | 270  | 280 | 290 | 300 |  |
|      | 2dVL-TTCL native_cloned      | 251 | AGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTG | 300 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 251 | .....  | 300 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 245 | .....  | 294 |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 1   |     |     |  |
|      |                              | 310 | 320  | 330 | 340 | 350 |  |
|      | 2dVL-TTCL native_cloned      | 301 | TTCGGTGGGGGAACCAAAGTACTGTCCTAAGCCGAAGTGTGGCTGCACC  | 350 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 301 | .....  | 350 |     |     |  |
|      | 2d12.5 VL native hybridoma   | 295 | .....A.....G.....G..                               | 327 |     |     |  |
|      | TTCL template for gene assem | 1   | -----  | 17  |     |     |  |
|      |                              | 360 | 370  | 380 | 390 | 400 |  |
|      | 2dVL-TTCL native_cloned      | 351 | ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGT  | 400 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 351 | .....  | 400 |     |     |  |
|      | 2d12.5 VL native hybridoma   |     | .....  |     |     |     |  |
|      | TTCL template for gene assem | 18  | .....  | 67  |     |     |  |
|      |                              | 410 | 420  | 430 | 440 | 450 |  |
|      | 2dVL-TTCL native_cloned      | 401 | CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTA  | 450 |     |     |  |
|      | 2dVL-TTCL N53C_cloned        | 401 | .....  | 450 |     |     |  |
|      | 2d12.5 VL native hybridoma   |     | .....  |     |     |     |  |
|      | TTCL template for gene assem | 68  | .....  | 117 |     |     |  |



FIG. 7B

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

|                              |     |   |  |     |     |     |  |
|------------------------------|-----|---|--|-----|-----|-----|--|
|                              |     | 460                                       | 470  | 480 | 490 | 500 |  |
| 2dVL-TTCL native_cloned      | 451 | ..... ..... ..... ..... ..... ..... ..... | CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT | 500 |     |     |  |
| 2dVL-TTCL N53C_cloned        | 451 | ..... ..... ..... ..... ..... ..... ..... | .....  | 500 |     |     |  |
| 2d12.5 VL native hybridoma   |     |   |  |     |     |     |  |
| TTCL template for gene assem | 118 | ..... ..... ..... ..... ..... ..... ..... | .....  | 167 |     |     |  |
|                              |     | 510                                       | 520  | 530 | 540 | 550 |  |
| 2dVL-TTCL native_cloned      | 501 | ..... ..... ..... ..... ..... ..... ..... | CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGA  | 550 |     |     |  |
| 2dVL-TTCL N53C_cloned        | 501 | ..... ..... ..... ..... ..... ..... ..... | .....  | 550 |     |     |  |
| 2d12.5 VL native hybridoma   |     |   |  |     |     |     |  |
| TTCL template for gene assem | 168 | ..... ..... ..... ..... ..... ..... ..... | .....  | 217 |     |     |  |
|                              |     | 560                                       | 570  | 580 | 590 | 600 |  |
| 2dVL-TTCL native_cloned      | 551 | ..... ..... ..... ..... ..... ..... ..... | CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC | 600 |     |     |  |
| 2dVL-TTCL N53C_cloned        | 551 | ..... ..... ..... ..... ..... ..... ..... | .....  | 600 |     |     |  |
| 2d12.5 VL native hybridoma   |     |   |  |     |     |     |  |
| TTCL template for gene assem | 218 | ..... ..... ..... ..... ..... ..... ..... | .....  | 267 |     |     |  |
|                              |     | 610                                       | 620  | 630 | 640 | 650 |  |
| 2dVL-TTCL native_cloned      | 601 | ..... ..... ..... ..... ..... ..... ..... | ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA | 650 |     |     |  |
| 2dVL-TTCL N53C_cloned        | 601 | ..... ..... ..... ..... ..... ..... ..... | .....T.....  | 650 |     |     |  |
| 2d12.5 VL native hybridoma   |     |   |  |     |     |     |  |
| TTCL template for gene assem | 268 | ..... ..... ..... ..... ..... ..... ..... | .....  | 317 |     |     |  |
|                              |     | 660                                       |  |     |     |     |  |
| 2dVL-TTCL native_cloned      | 651 | ..... ..... .....                         | GTGTTAATTCTAGA                                     | 664 |     |     |  |
| 2dVL-TTCL N53C_cloned        | 651 | ..... ..... .....                         | .....  | 664 |     |     |  |
| 2d12.5 VL native hybridoma   |     |   |  |     |     |     |  |
| TTCL template for gene assem | 318 | ..... ..... .....                         | .....  | 322 |     |     |  |

FIG. 8

## Translation of Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

|      |                             |     |                     |                   |                |            |             |     |
|------|-----------------------------|-----|---------------------|-------------------|----------------|------------|-------------|-----|
|      |                             |     | 10                  | 20                | 30             | 40         | 50          |     |
| (35) | 2dVH-TTCH_native cloned     | 1   | RSVKLQESGPGLVQPSQSL | SITCTVSGFSLTDYGVH | WVRQSPGKGLEWLG |            |             | 50  |
| (36) | 2dVH-TTCH_N87D_clon d       | 1   | .....               | .....             | .....          | .....      |             | 50  |
| (37) | 2dVH-TTCH_N87D_G53C_cloned  | 1   | .....               | .....             | .....          | .....      |             | 50  |
| (38) | 2dVH-TTCH_N87D_G54C_cloned  | 1   | .....               | .....             | .....          | .....      |             | 50  |
| (39) | 2dVH-TTCH_N87D_G55C_cloned  | 1   | .....               | .....             | .....          | .....      |             | 50  |
| (40) | 2dVH-TTCH expected sequence | 1   | .....               | .....             | .....          | .....      |             | 50  |
| (41) | 2d12.5 VH native hybridoma  | 1   | .....               | .....             | .....          | .....      |             | 48  |
|      |                             |     | 60                  | 70                | 80             | 90         | 100         |     |
|      | 2dVH-TTCH_native cloned     | 51  | VIWSGGGTAYTA        | AFISRLNIYKD       | NSKNQVFFEM     | NSLQANDTAM | YYCARRG     | 100 |
|      | 2dVH-TTCH_N87D_cloned       | 51  | .....               | .....             | .....          | .....      | D.....      | 100 |
|      | 2dVH-TTCH_N87D_G53C_cloned  | 51  | .....C.....         | .....             | .....          | .....      | D.....      | 100 |
|      | 2dVH-TTCH_N87D_G54C_cloned  | 51  | .....C.....         | .....             | .....          | .....      | D.....      | 100 |
|      | 2dVH-TTCH_N87D_G55C_cloned  | 51  | .....C.....         | .....             | .....          | .....      | D.....      | 100 |
|      | 2dVH-TTCH expected sequence | 51  | .....               | .....             | .....          | .....      | .....       | 100 |
|      | 2d12.5 VH native hybridoma  | 49  | .....               | .....             | .....          | .....      | .....       | 98  |
|      |                             |     | 110                 | 120               | 130            | 140        | 150         |     |
|      | 2dVH-TTCH_native cloned     | 101 | SYPYNYFDVWG         | QGTTVTVSAA        | STKGPSVFPL     | APSSKSTSG  | GTAALGCLVK  | 150 |
|      | 2dVH-TTCH_N87D_cloned       | 101 | .....               | .....             | .....          | .....      | .....       | 150 |
|      | 2dVH-TTCH_N87D_G53C_cloned  | 101 | .....               | .....             | .....          | .....      | .....       | 150 |
|      | 2dVH-TTCH_N87D_G54C_cloned  | 101 | .....               | .....             | .....          | .....      | .....       | 150 |
|      | 2dVH-TTCH_N87D_G55C_cloned  | 101 | .....               | .....             | .....          | .....      | .....       | 150 |
|      | 2dVH-TTCH expected sequence | 101 | .....               | .....             | .....          | .....      | .....       | 150 |
|      | 2d12.5 VH native hybridoma  | 99  | .....               | .....S            | .....          | .....      | .....       | 118 |
|      |                             |     | 160                 | 170               | 180            | 190        | 200         |     |
|      | 2dVH-TTCH_native cloned     | 151 | DYFPEPVTVSW         | NSGALTSGV         | HTFPAVLQSS     | GLYSLSSV   | TVPSSSLGTQT | 200 |
|      | 2dVH-TTCH_N87D_cloned       | 151 | .....               | .....             | .....          | .....      | .....       | 200 |
|      | 2dVH-TTCH_N87D_G53C_cloned  | 151 | .....               | .....             | .....          | .....      | .....       | 200 |
|      | 2dVH-TTCH_N87D_G54C_cloned  | 151 | .....               | .....             | .....          | .....      | .....       | 200 |
|      | 2dVH-TTCH_N87D_G55C_cloned  | 151 | .....               | .....             | .....          | .....      | .....       | 200 |
|      | 2dVH-TTCH expected sequence | 151 | .....               | .....             | .....          | .....      | .....       | 200 |
|      | 2d12.5 VH native hybridoma  |     |                     |                   |                |            |             |     |
|      |                             |     | 210                 | 220               |                |            |             |     |
|      | 2dVH-TTCH_native cloned     | 201 | YICNVNHKPS          | NTKVDRKAE         | PKSCDKSR       |            |             | 227 |
|      | 2dVH-TTCH_N87D_cloned       | 201 | .....               | .....             | .....          |            |             | 227 |
|      | 2dVH-TTCH_N87D_G53C_cloned  | 201 | .....               | .....             | .....          |            |             | 227 |
|      | 2dVH-TTCH_N87D_G54C_cloned  | 201 | .....               | .....             | .....          |            |             | 227 |
|      | 2dVH-TTCH_N87D_G55C_cloned  | 201 | .....               | .....             | .....          |            |             | 227 |
|      | 2dVH-TTCH expected sequence | 201 | .....               | .....             | .....          |            |             | 227 |
|      | 2d12.5 VH native hybridoma  |     |                     |                   |                |            |             |     |

FIG. 9A

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

|                                  |     |   |     |     |     |     |  |
|----------------------------------|-----|---|-----|-----|-----|-----|--|
|                                  |     | 10  | 20  | 30  | 40  | 50  |  |
| (42) 2dVH-TTCH_native cloned     | 1   | AGATCTGTGAAGCTGCAGGAGTCTGGACCTGGCCTAGTGCAGCCCTCACA    | 50  |     |     |     |  |
| (43) 2dVH-TTCH_N87D_cloned       | 1   | .....   | 50  |     |     |     |  |
| (44) 2dVH-TTCH_N87D_G53C_cloned  | 1   | .....   | 50  |     |     |     |  |
| (45) 2dVH-TTCH_N87D_G54C_cloned  | 1   | .....G.....   | 50  |     |     |     |  |
| (46) 2dVH-TTCH_N87D_G55C_cloned  | 1   | .....   | 50  |     |     |     |  |
| (47) 2dVH-TTCH expected sequence | 1   | .....   | 50  |     |     |     |  |
| (48) 2d12.5 VH native hybridoma  | 1   | .....A.....   | 44  |     |     |     |  |
|                                  |     | 60  | 70  | 80  | 90  | 100 |  |
| 2dVH-TTCH_native cloned          | 51  | GAGCCTGTCCATCACCTGCACGGTCTCTGGTTTCTCATTAAGTACTGACTATG | 100 |     |     |     |  |
| 2dVH-TTCH_N87D_cloned            | 51  | .....   | 100 |     |     |     |  |
| 2dVH-TTCH_N87D_G53C_cloned       | 51  | .....   | 100 |     |     |     |  |
| 2dVH-TTCH_N87D_G54C_cloned       | 51  | .....   | 100 |     |     |     |  |
| 2dVH-TTCH_N87D_G55C_cloned       | 51  | .....   | 100 |     |     |     |  |
| 2dVH-TTCH expected sequence      | 51  | .....   | 100 |     |     |     |  |
| 2d12.5 VH native hybridoma       | 45  | .....   | 94  |     |     |     |  |
|                                  |     | 110   | 120 | 130 | 140 | 150 |  |
| 2dVH-TTCH_native cloned          | 101 | GTGTACACTGGGTTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGA   | 150 |     |     |     |  |
| 2dVH-TTCH_N87D_cloned            | 101 | .....   | 150 |     |     |     |  |
| 2dVH-TTCH_N87D_G53C_cloned       | 101 | .....   | 150 |     |     |     |  |
| 2dVH-TTCH_N87D_G54C_cloned       | 101 | .....   | 150 |     |     |     |  |
| 2dVH-TTCH_N87D_G55C_cloned       | 101 | .....   | 150 |     |     |     |  |
| 2dVH-TTCH expected sequence      | 101 | .....   | 150 |     |     |     |  |
| 2d12.5 VH native hybridoma       | 95  | .....   | 144 |     |     |     |  |
|                                  |     | 160   | 170 | 180 | 190 | 200 |  |
| 2dVH-TTCH_native cloned          | 151 | GTGATATGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAG    | 200 |     |     |     |  |
| 2dVH-TTCH_N87D_cloned            | 151 | .....   | 200 |     |     |     |  |
| 2dVH-TTCH_N87D_G53C_cloned       | 151 | .....T.....   | 200 |     |     |     |  |
| 2dVH-TTCH_N87D_G54C_cloned       | 151 | .....T.T.....   | 200 |     |     |     |  |
| 2dVH-TTCH_N87D_G55C_cloned       | 151 | .....T.....   | 200 |     |     |     |  |
| 2dVH-TTCH expected sequence      | 151 | .....   | 200 |     |     |     |  |
| 2d12.5 VH native hybridoma       | 145 | .....   | 194 |     |     |     |  |
|                                  |     | 210   | 220 | 230 | 240 | 250 |  |
| 2dVH-TTCH_native cloned          | 201 | ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTCTTTGAAATGA     | 250 |     |     |     |  |
| 2dVH-TTCH_N87D_cloned            | 201 | .....   | 250 |     |     |     |  |
| 2dVH-TTCH_N87D_G53C_cloned       | 201 | .....   | 250 |     |     |     |  |
| 2dVH-TTCH_N87D_G54C_cloned       | 201 | .....   | 250 |     |     |     |  |
| 2dVH-TTCH_N87D_G55C_cloned       | 201 | .....   | 250 |     |     |     |  |
| 2dVH-TTCH expected sequence      | 201 | .....   | 250 |     |     |     |  |
| 2d12.5 VH native hybridoma       | 195 | .....   | 244 |     |     |     |  |
|                                  |     | 260   | 270 | 280 | 290 | 300 |  |
| 2dVH-TTCH_native cloned          | 251 | ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT    | 300 |     |     |     |  |
| 2dVH-TTCH_N87D_cloned            | 251 | .....G.....   | 300 |     |     |     |  |
| 2dVH-TTCH_N87D_G53C_cloned       | 251 | .....G.....   | 300 |     |     |     |  |
| 2dVH-TTCH_N87D_G54C_cloned       | 251 | .....G.....   | 300 |     |     |     |  |
| 2dVH-TTCH_N87D_G55C_cloned       | 251 | .....G.....   | 300 |     |     |     |  |
| 2dVH-TTCH expected sequence      | 251 | .....   | 300 |     |     |     |  |
| 2d12.5 VH native hybridoma       | 245 | .....   | 294 |     |     |     |  |
|                                  |     | 310   | 320 | 330 | 340 | 350 |  |
|                                  |     | .....   |     |     |     |     |  |

FIG. 9B

## Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

|   |     |  |     |
|---|-----|--|-----|
| 2dVH-TTCH_native cloned   | 301 | AGCTACCCCTTACAACCTACTTCGATGTCTGGGGCCAAGGGACCACGGTCAC | 350 |
| 2dVH-TTCH_N87D_cloned   | 301 | .....  | 350 |
| 2dVH-TTCH_N87D_G53C_cloned  | 301 | .....  | 350 |
| 2dVH-TTCH_N87D_G54C_cloned  | 301 | .....  | 350 |
| 2dVH-TTCH_N87D_G55C_cloned  | 301 | .....  | 350 |
| 2dVH-TTCH expected sequence   | 301 | .....  | 350 |
| 2d12.5 VH native hybridoma  | 295 | .....A.....  | 344 |
| <div> <div>360370380390400</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 351 | CGTCTCCGCAGCCTCCACCAAGGGGCCCATCGGTCTTCCCCCTGGCACCCT  | 400 |
| 2dVH-TTCH_N87D_cloned   | 351 | .....  | 400 |
| 2dVH-TTCH_N87D_G53C_cloned  | 351 | .....  | 400 |
| 2dVH-TTCH_N87D_G54C_cloned  | 351 | .....  | 400 |
| 2dVH-TTCH_N87D_G55C_cloned  | 351 | .....  | 400 |
| 2dVH-TTCH expected sequence   | 351 | .....  | 400 |
| 2d12.5 VH native hybridoma  | 345 | .....T..   | 354 |
| <div> <div>410420430440450</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 401 | CCTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAG    | 450 |
| 2dVH-TTCH_N87D_cloned   | 401 | .....  | 450 |
| 2dVH-TTCH_N87D_G53C_cloned  | 401 | .....  | 450 |
| 2dVH-TTCH_N87D_G54C_cloned  | 401 | .....  | 450 |
| 2dVH-TTCH_N87D_G55C_cloned  | 401 | .....  | 450 |
| 2dVH-TTCH expected sequence   | 401 | .....  | 450 |
| 2d12.5 VH native hybridoma  |     |  |     |
| <div> <div>460470480490500</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 451 | GACTACTTCCCCGAACCGGTGACGGTGTCTTGGAACTCAGGCGCCCTGAC   | 500 |
| 2dVH-TTCH_N87D_cloned   | 451 | .....  | 500 |
| 2dVH-TTCH_N87D_G53C_cloned  | 451 | .....  | 500 |
| 2dVH-TTCH_N87D_G54C_cloned  | 451 | .....  | 500 |
| 2dVH-TTCH_N87D_G55C_cloned  | 451 | .....  | 500 |
| 2dVH-TTCH expected sequence   | 451 | .....G.....  | 500 |
| 2d12.5 VH native hybridoma  |     |  |     |
| <div> <div>510520530540550</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 501 | CAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACT     | 550 |
| 2dVH-TTCH_N87D_cloned   | 501 | .....  | 550 |
| 2dVH-TTCH_N87D_G53C_cloned  | 501 | .....  | 550 |
| 2dVH-TTCH_N87D_G54C_cloned  | 501 | .....  | 550 |
| 2dVH-TTCH_N87D_G55C_cloned  | 501 | .....  | 550 |
| 2dVH-TTCH expected sequence   | 501 | .....  | 550 |
| 2d12.5 VH native hybridoma  |     |  |     |
| <div> <div>560570580590600</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 551 | CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC   | 600 |
| 2dVH-TTCH_N87D_cloned   | 551 | .....  | 600 |
| 2dVH-TTCH_N87D_G53C_cloned  | 551 | .....  | 600 |
| 2dVH-TTCH_N87D_G54C_cloned  | 551 | .....  | 600 |
| 2dVH-TTCH_N87D_G55C_cloned  | 551 | .....  | 600 |
| 2dVH-TTCH expected sequence   | 551 | .....  | 600 |
| 2d12.5 VH native hybridoma  |     |  |     |
| <div> <div>610620630640650</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div> |     |  |     |
| 2dVH-TTCH_native cloned   | 601 | TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA   | 650 |
| 2dVH-TTCH_N87D_cloned   | 601 | .....  | 650 |

FIG. 9C

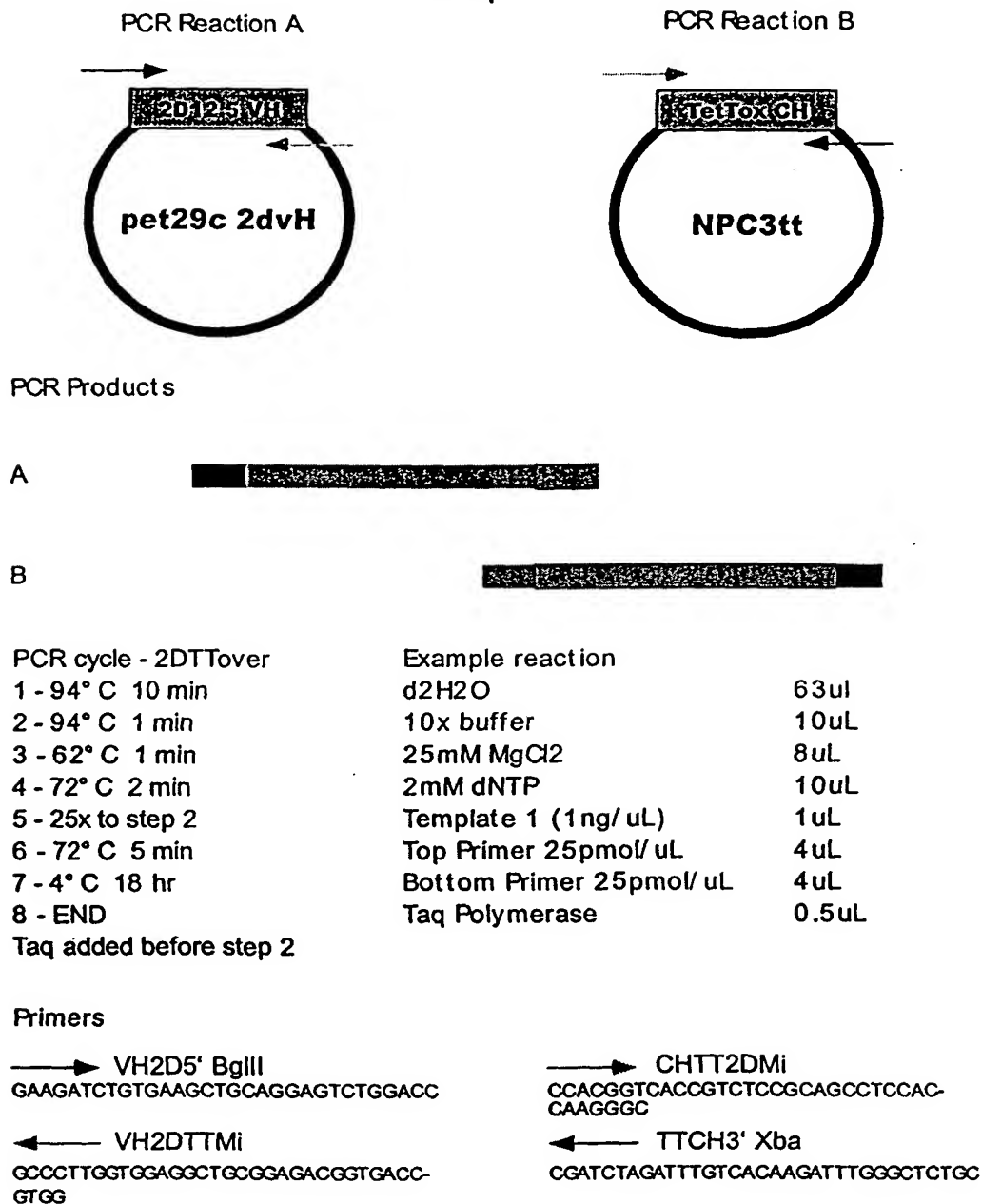
## Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

|                             |     |       |     |
|-----------------------------|-----|-------|-----|
| 2dVH-TTCH N87D_G53C_cloned  | 601 | ..... | 650 |
| 2dVH-TTCH N87D_G54C_cloned  | 601 | ..... | 650 |
| 2dVH-TTCH N87D_G55C_cloned  | 601 | ..... | 650 |
| 2dVH-TTCH expected sequence | 601 | ..... | 650 |
| 2d12.5 VH native hybridoma  |     |       |     |

|                             |     |                                 |     |     |
|-----------------------------|-----|---------------------------------|-----|-----|
|                             |     | 660                             | 670 | 680 |
|                             |     | .... .... .... .... .... .... . |     |     |
| 2dVH-TTCH_native cloned     | 651 | AGCAGAGCCCAAATCTTGTGACAAATCTAGA | 681 |     |
| 2dVH-TTCH_N87D_cloned       | 651 | .....                           | 681 |     |
| 2dVH-TTCH_N87D_G53C_cloned  | 651 | .....                           | 681 |     |
| 2dVH-TTCH_N87D_G54C_cloned  | 651 | .....                           | 681 |     |
| 2dVH-TTCH_N87D_G55C_cloned  | 651 | .....                           | 681 |     |
| 2dVH-TTCH expected sequence | 651 | .....                           | 681 |     |
| 2d12.5 VH native hybridoma  |     |                                 |     |     |

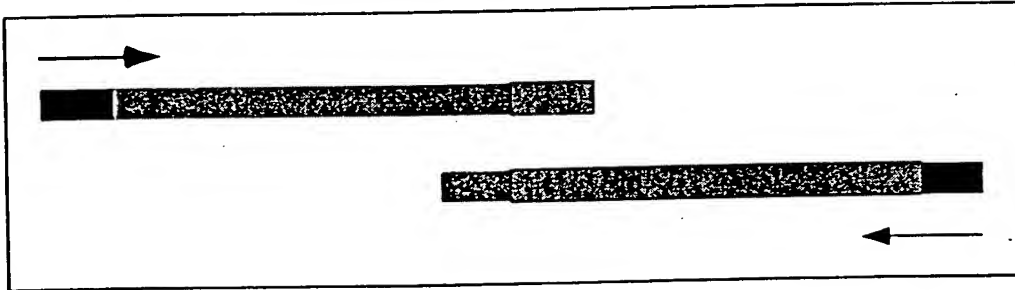
FIG. 10A

# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 1



# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 2

## PCR Reaction



### PCR cycle - 2DTTVent

- 1 - 95°C 10 min
  - 2 - 94°C 1 min
  - 3 - 60°C 1 min
  - 4 - 75°C 2 min
  - 5 - 4x to step 2
  - 6 - 94°C 1 min
  - 7 - 63°C 1 min
  - 8 - 75°C 2 min
  - 9 - 25x to step 6
  - 10 - 72°C 5 min
  - 11 - 4°C 18 hr
  - 12 - END
- Vent added before step 2  
Primers added before step 6

### Primers

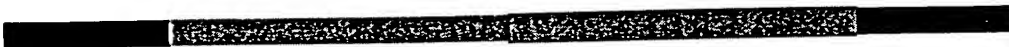
→ VH2D5' BglII  
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba  
CGATCTAGATTTGTCACAAGATTTGGGCTCTGC

### Example reaction

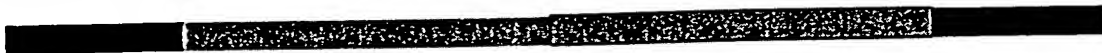
|                          |       |
|--------------------------|-------|
| d2H2O                    | 70uL  |
| 10x buffer               | 10uL  |
| 100mM MgSO4              | 0uL   |
| 2mM dNTP                 | 10uL  |
| Template 1(1ng/ uL)      | 1uL   |
| Template 2(1ng/ uL)      | 1uL   |
| Top Primer 25pmol/ uL    | 4uL   |
| Bottom Primer 25pmol/ uL | 4uL   |
| Vent Polymerase          | 0.5uL |

### PCR Assembly Product

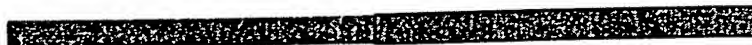


# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His  
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

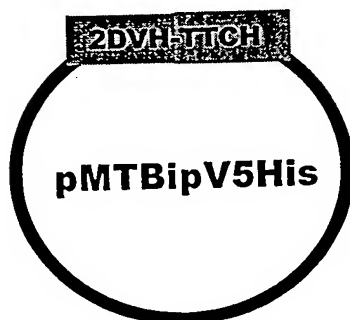
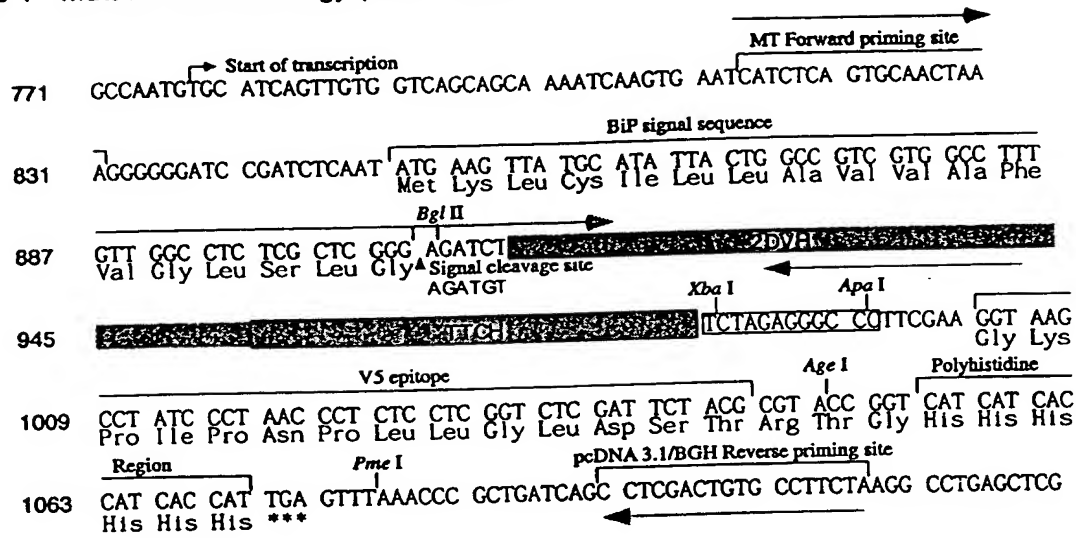


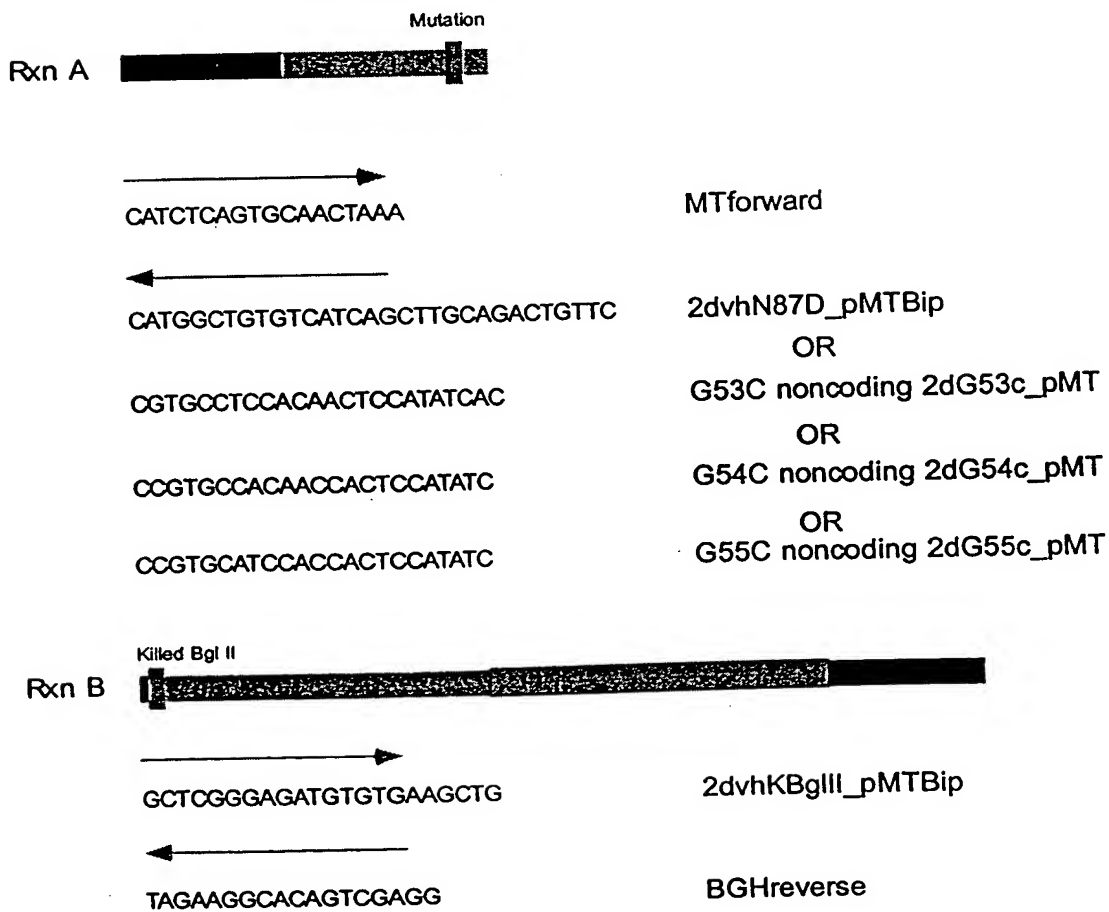


FIG. 10D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



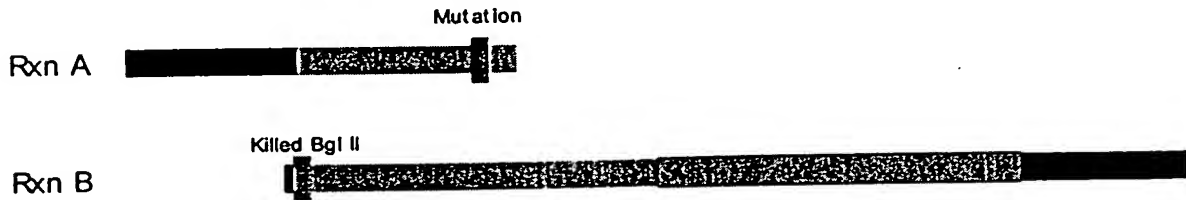
1st Set of PCR Reactions Product A and B)



Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction (Mix Products of reaction A and B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

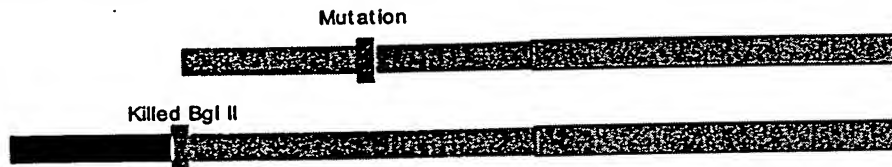


FIG. 10F

Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

- 1 - 95° C 10 min
- 2 - 94° C 1 min
- 3 - 50° C 1 min
- 4 - 75° C 2 min
- 5 - 24x to step 2
- 6 - 75° C 5 min
- 7 - 4° C 18 hr
- 8 - END

VENT added before step 2

Primers added before step 1

|                         |       |
|-------------------------|-------|
| Example reaction        |       |
| d2H2O                   | 70ul  |
| 10x buffer              | 10uL  |
| 100mM MgSO4             | 0uL   |
| 2mM dNTP                | 10uL  |
| Template (1ng/uL)       | 1uL   |
| Top Primer 25pmol/uL    | 4uL   |
| Bottom Primer 25pmol/uL | 4uL   |
| Vent Polymerase         | 0.5uL |

Step 2 - PCR Reaction VHMUTTAQ

PCR cycle - VHMUTTAQ

- 1 - 95° C 10 min
- 2 - 94° C 1 min
- 3 - 68° C 1 min
- 4 - 72° C 2 min
- 5 - 4x to step 2
- 6 - 94° C 1 min
- 7 - 50° C 1 min
- 8 - 72° C 2 min
- 9 - 24x to step 6
- 10 - 72° C 5 min
- 11 - 4° C 18 hr
- 12 - END

Taq added before step 2

Primers added before step 6

|                         |       |
|-------------------------|-------|
| Example reaction        |       |
| d2H2O                   | 61ul  |
| 10x buffer              | 10uL  |
| 25mM MgCl2              | 8uL   |
| 2mM dNTP                | 10uL  |
| Template 1 (1ng/uL)     | 1uL   |
| Template 2 (1ng/uL)     | 1uL   |
| Top Primer 25pmol/uL    | 4uL   |
| Bottom Primer 25pmol/uL | 4uL   |
| Taq Polymerase          | 0.5uL |

FIG. 11A

# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 1

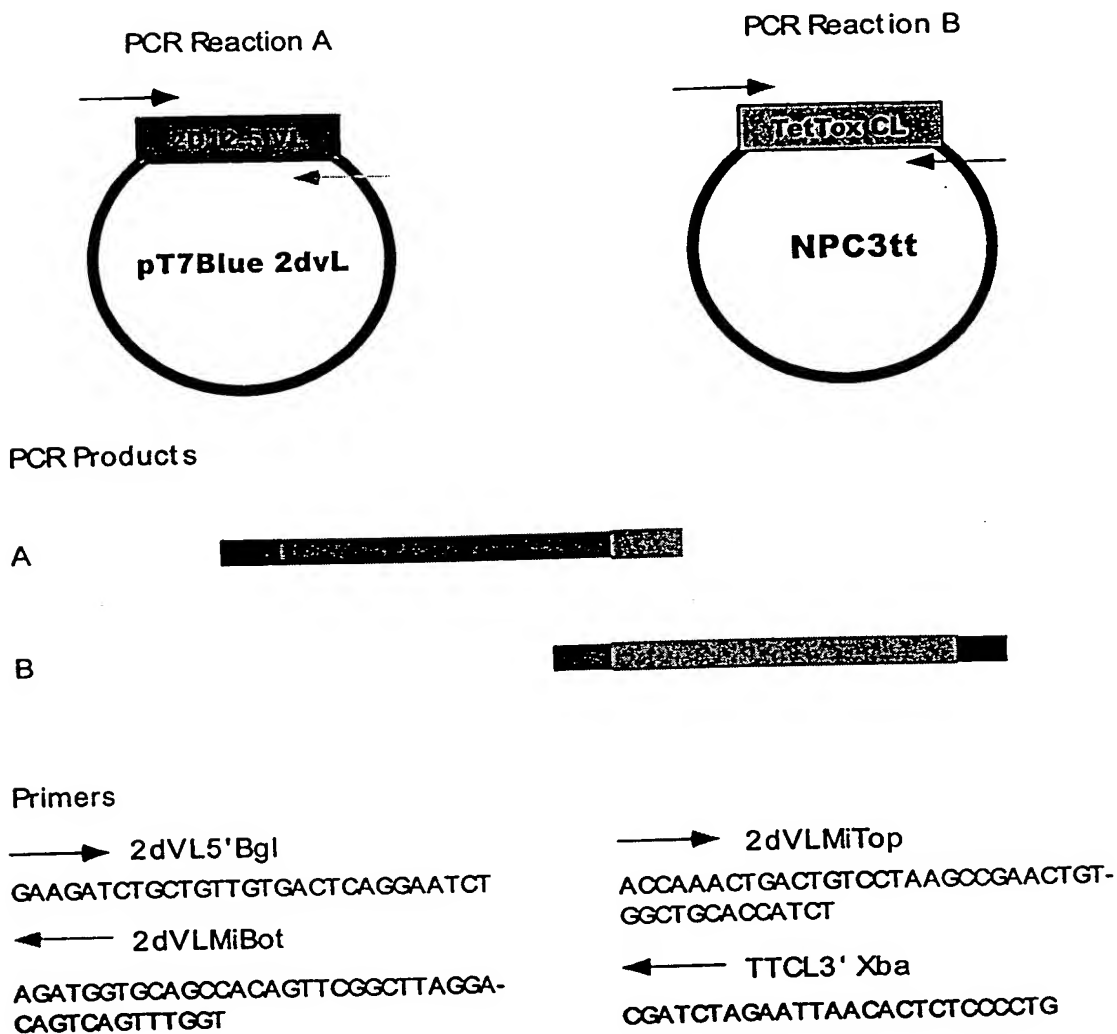
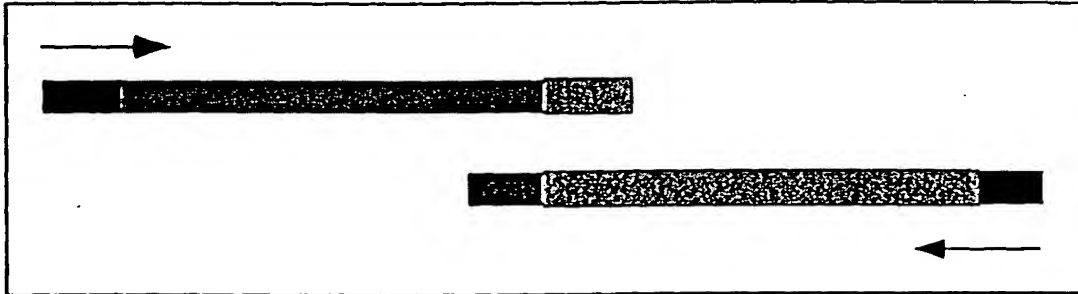


FIG. 11B

# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 2

## PCR Reaction



### Primers

→ 2dVL5' Bgl  
GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba  
CGATCTAGAATTAACACTCTCCCCTG

### PCR Assembly Product



## Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His  
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

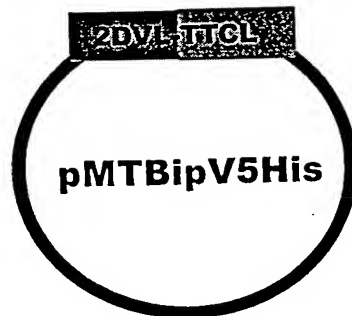
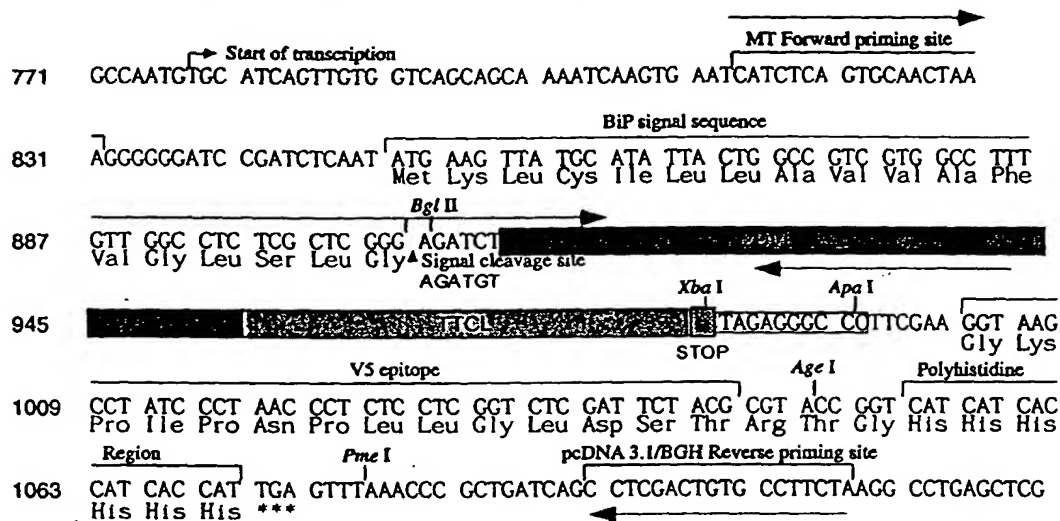


FIG. 11D

## Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



## 1st Set of PCR Reactions Producta A and B)

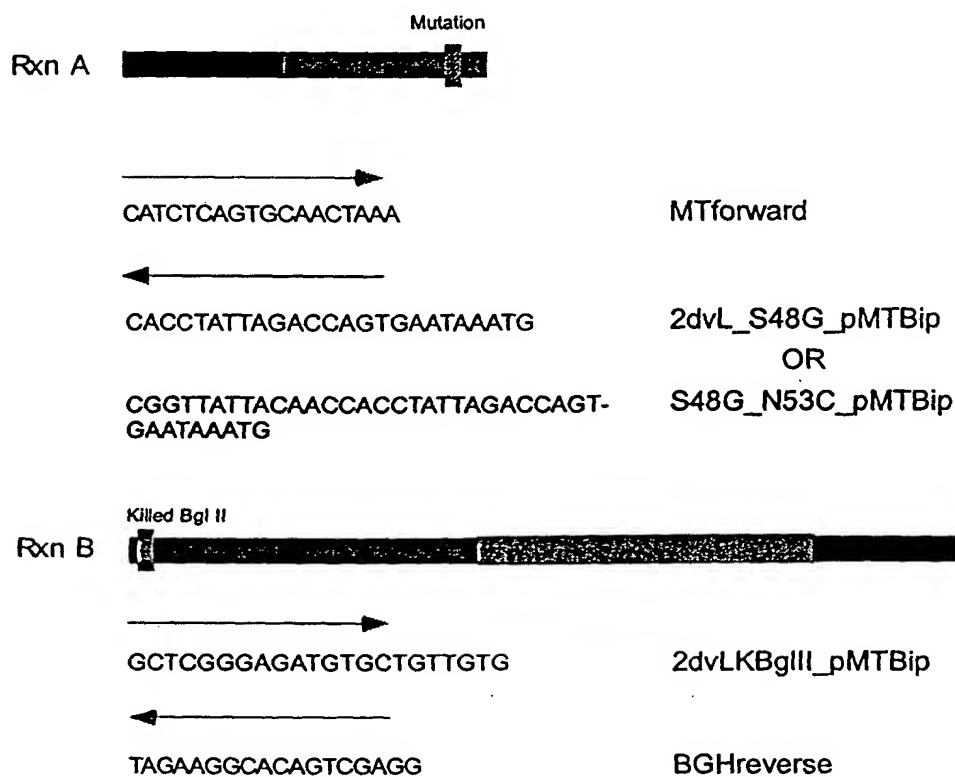


FIG. 11E

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction Mix Products of reaction B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)

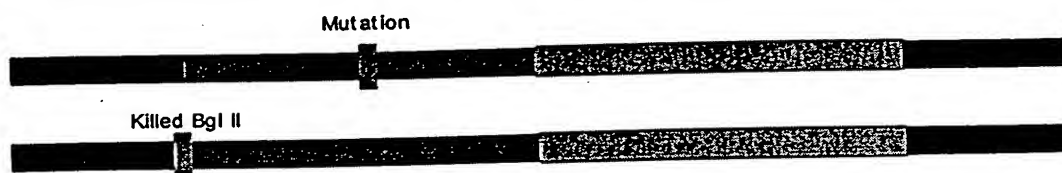
Rxn A



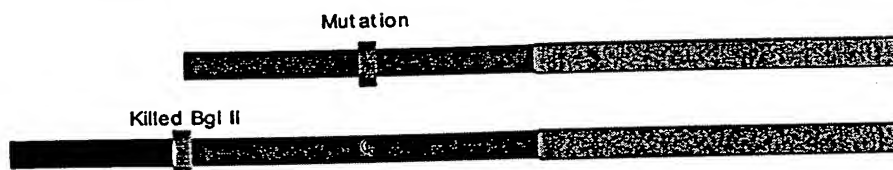
Rxn B



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1





# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 4

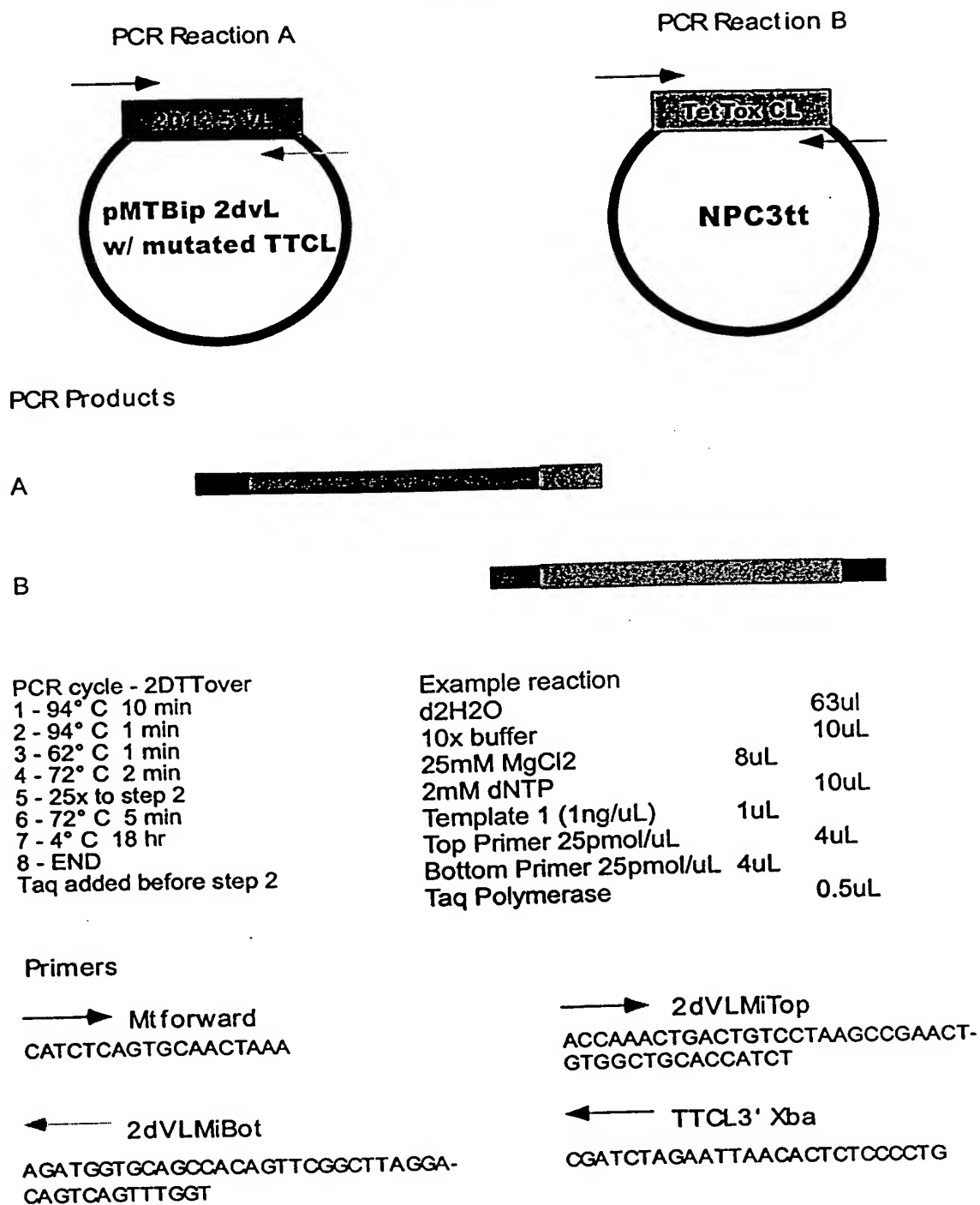


FIG. 11G

## Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected with the native light chain:

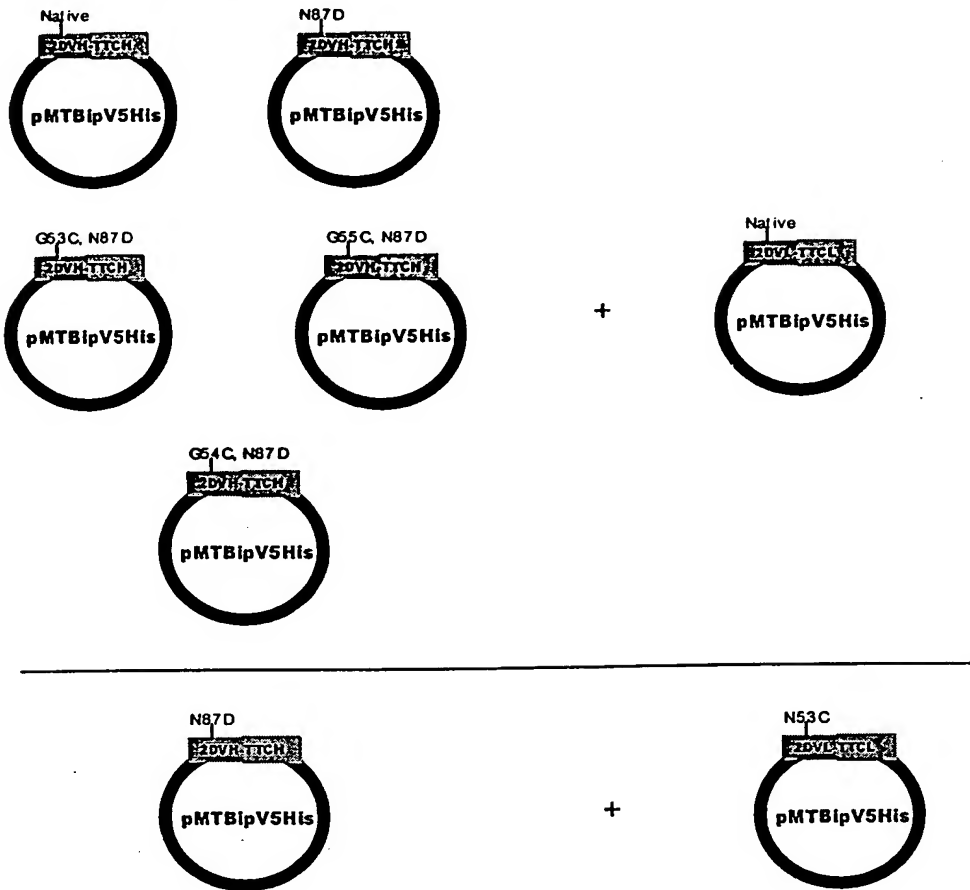


FIG. 12

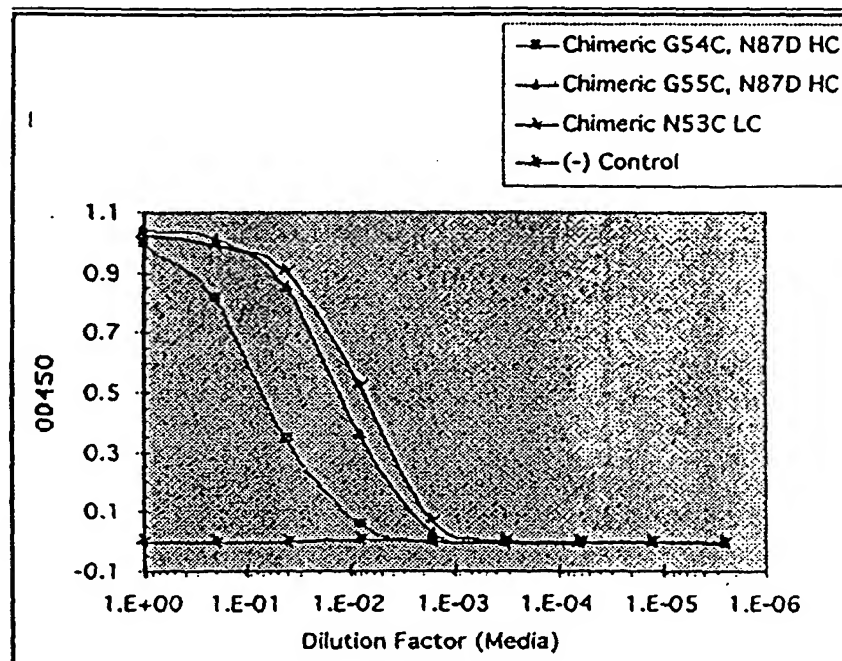
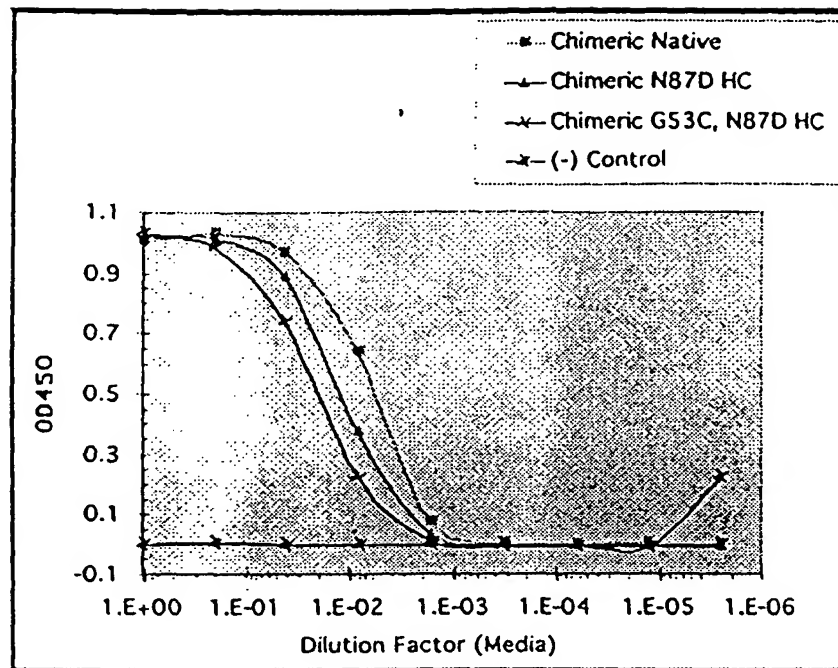


FIG. 13

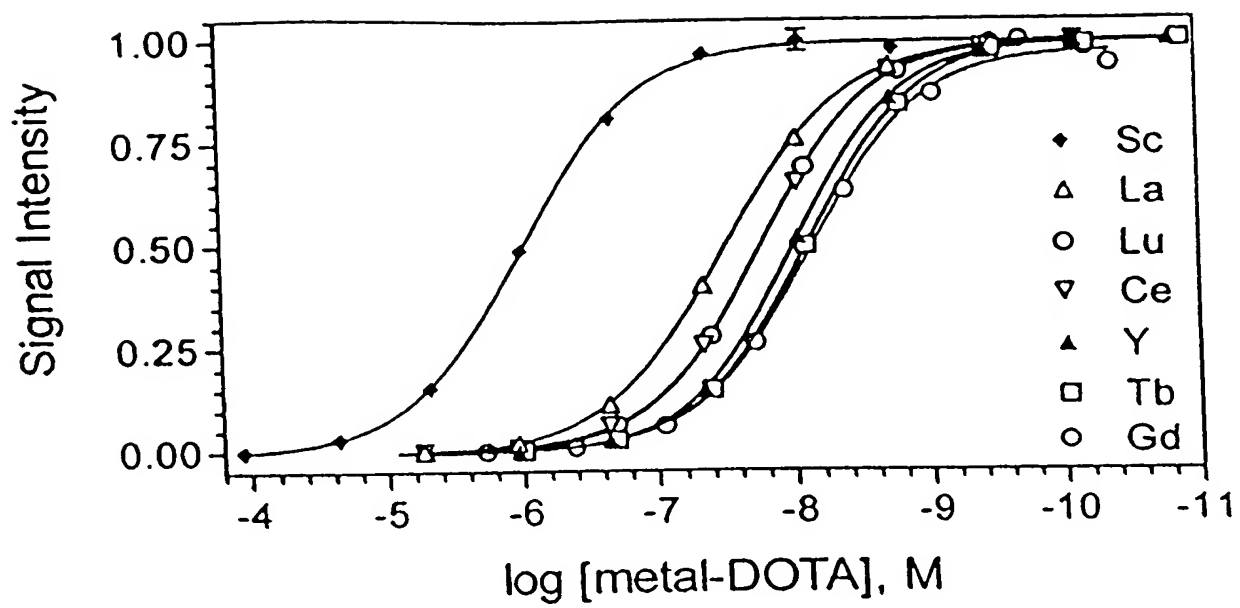


FIG. 14

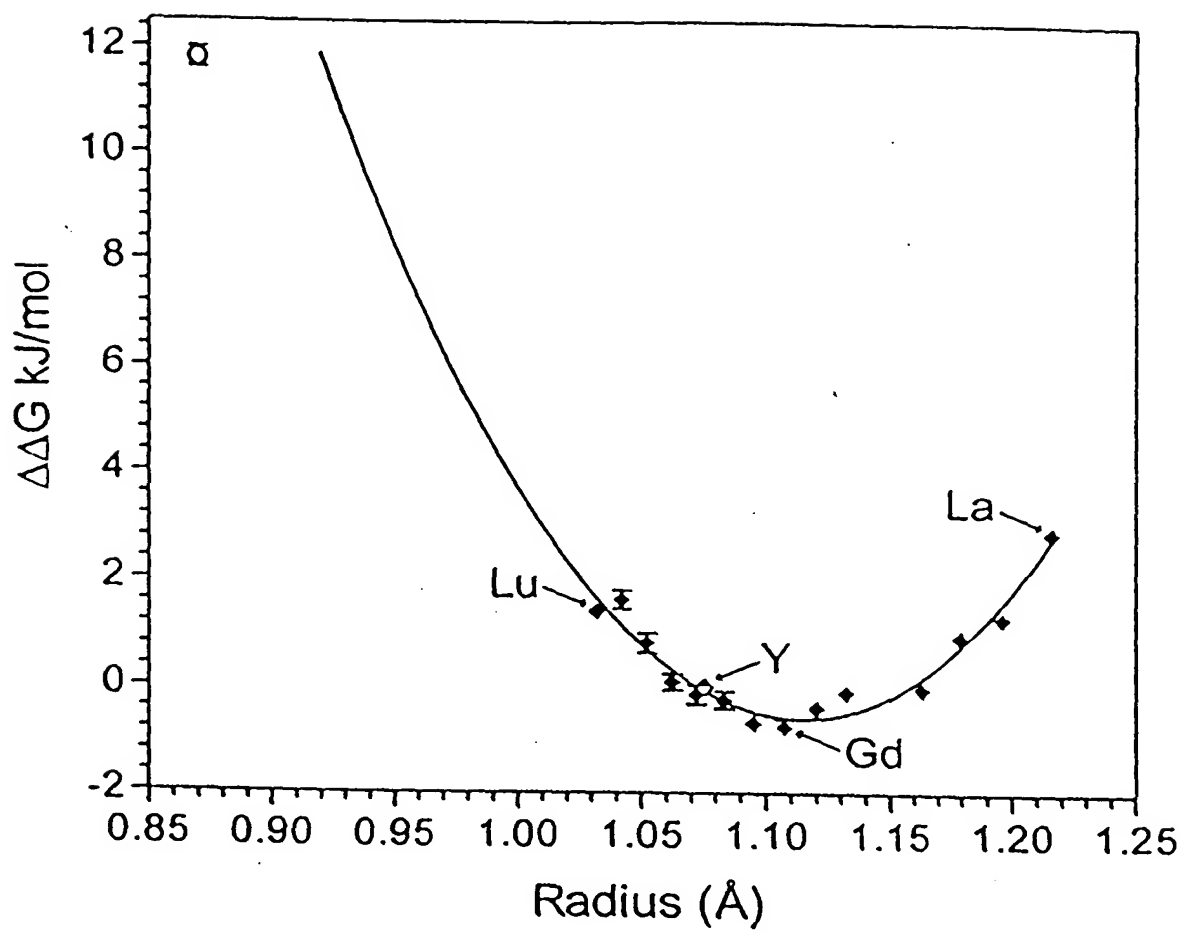


FIG. 15

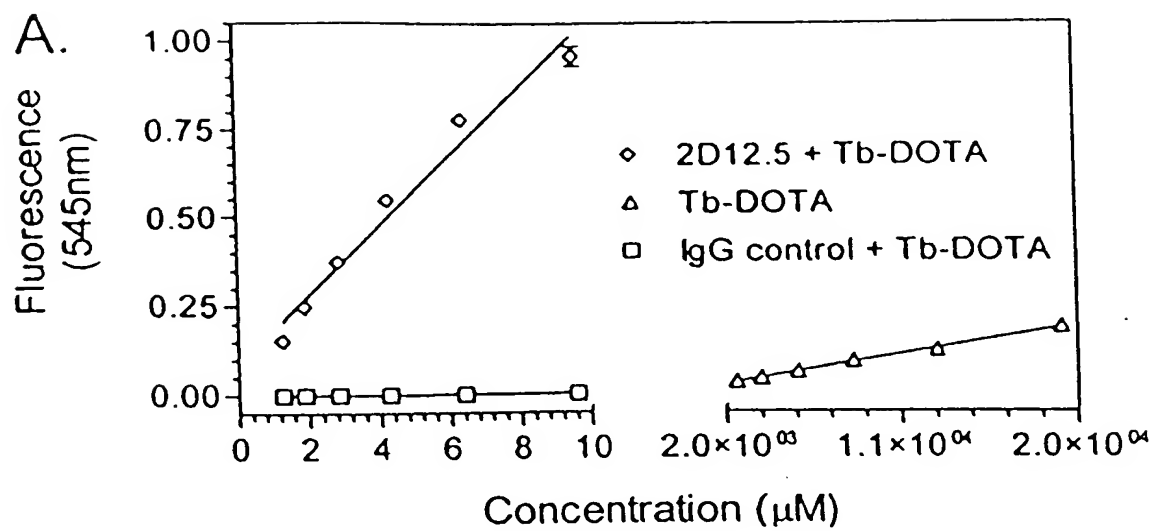


FIG. 16

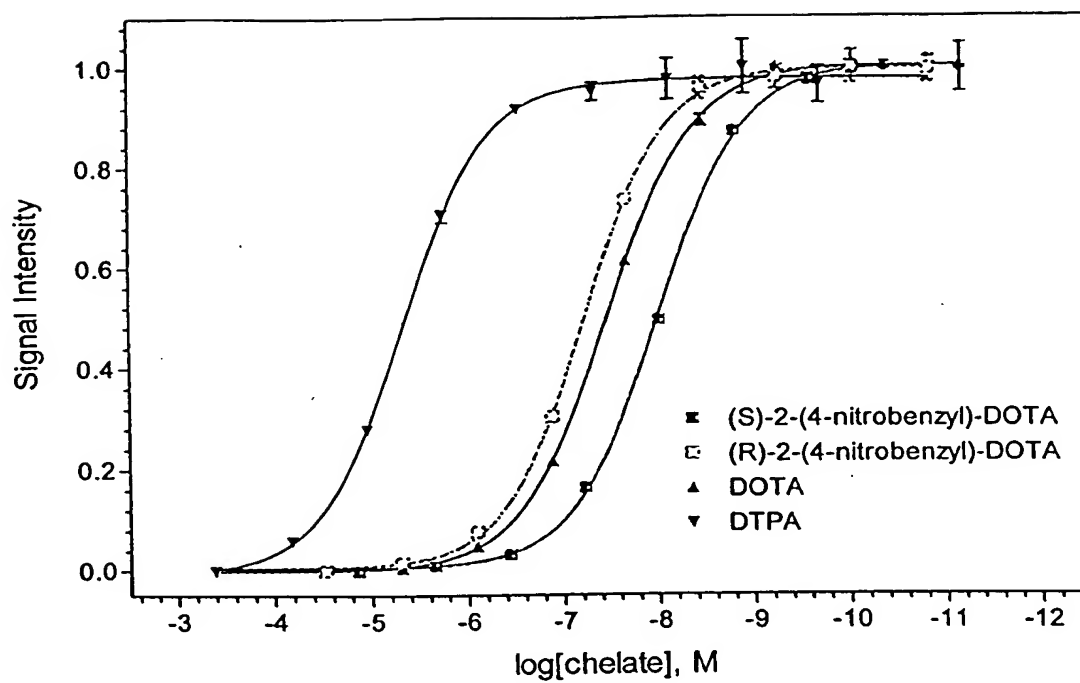


FIG. 17

